Anti-neutrophil cytoplasmic antibody (ANCA) testing at Groote Schuur Hospital: Adherence to indications for testing

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Declaration

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Dissertation Abstract

Anti-neutrophil cytoplasmic antibody (ANCA) testing at Groote Schuur Hospital: Adherence to indications for testing

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Introduction: Appropriate use of laboratory investigations is increasingly important in resource-constrained environments. We reviewed the anti-neutrophil cytoplasmic antibody (ANCA) testing practices in a tertiary hospital in South Africa.

Methods: We conducted a retrospective file review of all ANCA tests ordered through the National Health Laboratory Services (NHLS) at Groote Schuur Hospital over 12 months (01/01/17 – 31/12/17), including both inpatient and outpatients, and extracted sociodemographic and clinical details. All requests were assessed against the International Consensus Statement of 1999 which provides clinical guidelines for the indications for ANCA testing.

Results: Of the 945 ANCA tests requested, 790 patient records were reviewed, 62 patients had multiple tests and 155 patient records were missing. Most tests (63.5%) were performed on inpatients. Only 193 patients (24.4%) had indications for ANCA

testing meeting guidelines. The commonest non-guideline indications were critical limb ischemia (9.6%), stroke (7.3%), uveitis (5.7%), renal impairment (4.9%) and interstitial lung disease (4.4%). The departments for requesting ANCA tests most commonly were Medicine, Ophthalmology, Neurology and Surgery, with Surgery having 99% of its tests for non-guideline indications. Ten patients (1.3%) were diagnosed with ANCA-associated vasculitis (AAV), and of these 9 had renal-limited vasculitis. These patients were predominantly female (70.0%) with mean (SD) age of 54.5 (16.4) years. Twenty-six patients tested ANCA positive without any evidence of AAV. Of these false positives, ten (38.4%) were human immunodeficiency virus (HIV) positive, three (11.5%) had tuberculosis (TB), and three (11.5%) had other autoimmune diseases. The total estimated cost of ANCA tests for the year was ZAR274 046, with ZAR17 490 spent on duplicate testing and ZAR208 275 spent on non-indicated clinical conditions.

Conclusion: ANCA testing occurred outside standard guidelines in three-quarters of requests, and duplicate testing was common, with large cost implications. Chronic infections HIV and TB, and autoimmune conditions accounted for half of the false positive tests. We believe that training of clinicians and a gating policy will be cost-effective interventions.

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1. Professor Bridget Hodkinson

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List of tables and figures

Abbreviations

AAV	ANCA-associated vasculitis
ANCA	Anti-neutrophil cytoplasmic antibody
ANA	Antinuclear antibodies
C-ANCA	Cytoplasmic ANCA
CHCC	Chapel Hill Consensus Conference
EGPA	Eosinophilic granulomatosis with polyangiitis
ELISA	Enzyme-linked immunosorbent assay
FEIA	Fluorescent-enzyme immuno-assays
GSH	Groote Schuur Hospital
GPA	Granulomatosis with polyangiitis
GN	Glomerulonephritis
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disese
IgM	Immunoglobulin M

IgG	Immunoglobulin G
IIF	Indirect immunofluorescence
MPO	Myeloperoxidase
MPA	Microscopic polyangiitis
NHLS	National Health Laboratory Services
NPV	Negative predictive value
P-ANCA	Peri-nuclear ANCA
PPV	Positive predictive value
PR3	Proteinase 3
RA	Rheumatoid arthritis
RLV	Renal limited vasculitis
SLE	Systemic lupus erythematosus
SA	South Africa
TB	Tuberculosis

Chapter 1: Introduction and Literature Review

Context:

The rising cost of laboratory investigations and equitable distribution of health care resources have become important issues world-wide, with socio-political implications, particularly in resource constrained environments. However, health care workers often lack insight into the costs of tests and treatment, and frequently order tests without a deep understanding of the test's clinical utility. In any population, ANCA-associated vasculitis (AAV) is a rare multi-system condition that requires a high index of suspicion and testing within an appropriate clinical framework. This audit was undertaken to investigate the number of anti-neutrophil cytoplasmic antibody (ANCA) tests ordered over 12 months and review the adherence to international guidelines for these tests.

Background:

Anti-neutrophilic cytoplasmic antibody tests

Anti-neutrophil cytoplasmic antibody (ANCA) tests were first described in 1982 and are now integral to our understanding and classification of small vessel vasculitis [1]. The revised nomenclature in the 2012 Chapel Hill Consensus Conference (CHCC 2012) added a new category of vasculitis, the ANCA-associated vasculitis (AAV) which was defined as a necrotizing small vessel vasculitis with few or no immune deposits. The major clinicopathologic variants are granulomatosis with polyangiitis (GPA, previously known as Wegener's granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA, previously known as Churg-Strauss syndrome), and its localized forms (pauci-immune necrotizing and cresenteric glomerulonephritis [GN]) [2] (Table 1).

Anti-neutrophilic cytoplasmic antibodies are autoantibodies directed against antigens found in the cytoplasmic granules of neutrophils and monocytes [3]. Two types of ANCA patterns are identified in small vessel vasculitis using indirect immunofluorescence (IIF): cytoplasmic ANCA (c-ANCA) directed against proteinase 3 (PR3), and peri-nuclear ANCA (p-ANCA) targeting myeloperoxidase (MPO) [4]. In recognition of the important role of ANCA serology in these diseases, CHCC 2012 called for adding a prefix to the clinicopathologic phenotype indicating ANCA specificity as either MPO-ANCA or PR3-ANCA or ANCA negative [2]. These auto-antigens have different genetics and correlate better with different human leukocyte antigen (HLA) risk genes than its clinicopathological variants [5].

The manifestations, clinical course and relapse rates of AAV correlate closely with target antigen [6] [7]. Ear, nose, eye and lung involvement appear to occur more in patients with c-ANCA/ PR3 positivity, with renal manifestation to a lesser degree. This group also tends to have more relapses with a poorer outcome and higher mortality [8, 9]. c-ANCA occur in 95% of cases of new onset GPA and 40% of EGPA [4]. In comparison, the p-ANCA/ MPO group display more renal involvement, typically a pauci-immune segmental necrotizing GN, which could occur alone or with systemic features, and fewer relapses [10]. Positive p-ANCA are seen in in 80% of new onset MPA [4]. The target antigen has also been shown to have histological implications on the type of GN found at renal biopsy. Anti-PR3-AAV is associated with more granulomatous inflammation and necrosis while anti-MPO-AAV has a lesser degree of inflammation and more sclerosis [11].

With time and a wide variety of ANCA testing in formalin and ethanol fixed neutrophils, other patterns of ANCA have been identified including nuclear ANCA, atypical ANCA and double-positive p-ANCA and c-ANCA, making the need for more specific ANCA assays and guidelines for testing vital [12]. The international consensus statement guidelines (1999) showed that the combination of IIF and ELISA tests offered good clinical utility [13, 14]. Ten percent of ANCAs were detectable by IIF while remaining negative by first-generation ELISAs, while in 5% of AAV cases only the ELISA was positive with negative IIF [14]. This discrepancy highlighted the need for combination testing or an ELISA test if AAV was highly suspected despite a negative screening IIF. The combined use of IIF and first-generation ANCA ELISAs resulted in an increase in the diagnostic performance from a specificity of 76% to 98% respectively [14]. However, despite this recommendation, the concern in the clinical setting was over the delay in diagnosis and increased costs of these tests.

In 2017, Bossuyt and colleagues discussed the newer and more improved technologies for ANCA testing; with second and third generation ELISAs including direct and capture ELISAs, as well as new fluorescent-enzyme immuno-assays(FEIA), for PR3- and MPO-ANCA detection. These tests were again shown outperforming the classic IIF. They proposed a new strategy for using ELISA as a first line screening in patients suspected with AAV from the 1999 guideline scenarios. A follow up second assay, like IIF, could be used to increase sensitivity if ELISA was negative. They also opened the floor to using newer confirmatory immunoassays as long as they were internally validated [15]. Again, clinicians were told not to forget that the gold standard for diagnosis of AAV still remained biopsy of

the affected organ in the setting of a high clinical suspicion and negative immunoassay testing. This integral point had been highlighted previously by Damoiseaux *et al* in 2005 who demonstrated 10 out of 87 patients who were capture ELISA negative despite being confirmed with AAV by suggestive renal histology with a pauci-immune necrotising GN [16].

False positive anti-neutrophil cytoplasmic antibody tests

A positive ANCA test is not a definitive diagnostic indicator of AAV with its presence found in non-vasculitic diseases. While in 2002, Mandl *et al* demonstrated a false positive rate around 2.2%, mainly p-ANCA and anti-MPO antibodies [17]. More recent audits in Greece [18], Netherlands [19], India [20] and Canada [21] reported higher rates of 80%, 50%, 85% and 71% respectively. Non-vasculitic diseases include inflammatory bowel disease (up to 70% ANCA positivity in ulcerative colitis, 30% in Crohns disease), primary sclerosing cholangitis (90%), chronic autoimmune hepatitis (70%), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (20%) [3]. Edgar and McMillan showed that 18.2% ANCA positivity was due to RA and that 27.9% in itself accounted for the connective tissue disorders as a whole, making it another source of false positives [22].

False-positive ANCA tests have also been documented in the setting of chronic infection such as tuberculosis (TB), leprosy, suppurative lung disease [23], and amoebic liver abscess[24]. Among 60 West Africans, 6.7% of patients with TB and malaria were found to be ANCA positive by IIF and demonstrated positive IgM antibodies to MPO [25]. In cases of infective endocarditis, patients may present with multi-system disturbances resembling an autoimmune process, with a positive ANCA

and making diagnosis even more uncertain and less straightforward. This is important as distinguishing an infective from an autoimmune process is needed to guide appropriate therapy [25]. There has also been an association of recurrent infections with dual anti-PR3 and anti-MPO positivity, with no indication of any particular pattern of clinical outcome [26].

Anecdotal reports of associations between ANCA antibodies and viral infections including HIV, hepatitis B and C, have been noted [27]. HIV infection may evoke ANCA in 20 to 83% of cases, mainly positive by IIF and a small percentage by ELISA [27] [28]. Cornely *et al* found anti-MPO positivity in only one out of 199 HIV patients (0.5%) [29], whereas Koderisch *et al* found a faint c-ANCA positivity in 24 out of 29 (83%) HIV-infected patients [30]. Although many of these studies have been performed in a pre-highly active antiretroviral therapy (HAART) era, Iordache and colleagues showed the persistence of autoantibodies in active HIV despite good virologic control. They demonstrated that 45% had at least one autoantibody present, especially antinuclear antibodies (ANA) (33%) and ANCA (13%), without clinically relevant disease [31].

However, with the advent of effective HAART and good viral and immunological control, there has been a higher emergence of autoimmune diseases in these patients with reconstituted immune systems [32]. Vasculitis associated with HIV has been reported in all stages of HIV infection [33]. ANCA shows a high sensitivity and specificity for systemic necrotizing vasculitis and pauci-immune cresenteric glomerulonephritis in patients with HIV [34]. In addition to infections, certain medications like anti-thyroid drugs, levamisole-adulterated cocaine, minocycline and

hydralazine may induce ANCA and associated small-vessel vasculitis. Propylthiouracil has also been linked as a common cause of drug induced ANCA positive vasculitis, expressing high levels of MPO ANCA [3, 23].

Indications for anti-neutrophil cytoplasmic antibody-associated vasculitis testing

Because of the high false positive rate for indiscriminate ANCA testing, the international consensus statement of 1999 provided clinical guidelines of indications for ANCA testing [14] (Table 2). The aim of these guidelines is to increase the positive predictive value (PPV) of ANCA testing by limiting the test to patients with clinical features suggestive of AAV [14] . It was demonstrated that ANCA exhibit a PPV of 54% and negative predictive value (NPV) of 99% in conventional clinical settings. Variabilities also existed across tests due to the prevalence of AAV in the population examined, implying that better predictive values could be seen in groups of patients with higher pretest probability [17]. Encouragingly, studies show that AAV is seldom missed when testing is restricted to indications meeting the 1999 clinical guidelines [14]. Mandl *et al* calculated a potential 27% decrease in false positive rates if strict adherence to the guidelines were maintained. Unfortunately, clinicians do not always adhere to these guidelines, with 35-65% of tests being requested without indication [20, 35]. Clinical audits from differing counties and populations are summarized in Table 3.

Anti-neutrophil cytoplasmic antibody titres

There are also fluctuations in ANCA titres with higher titres usually occurring at presentation and decreasing with treatment initiation and stabilization of disease. Rebound increase in titres were seen in about half of GPA patients with disease

relapse [16, 36]. Currently, we do not know the clinical significance of change in titres in predicting AAV relapse, nor which test best defines these relapses. It was noted in a meta-analysis study that a rise in ANCA titers or persistence of ANCA during remission of AAV only mildly predicts the risk of relapse, hence serial ANCA testing is not recommended at this stage [37].

Epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis testing

The pre-test probability of ANCA testing is also dependent on the epidemiology of AAV within the testing region. A higher prevalence will of course beget a higher predictive value. Anti-neutrophil cytoplasmic antibody-associated vasculitis is very uncommon, with a prevalence rate in Europe of 20 per million [38]. There is little information on the worldwide epidemiology and clinical features of these diseases. There are regional and ethnic differences in the clinical features of patients with AAV, with most of the information coming from the western caucasian populations [38]. In southern Europe, MPA is more common, compared to northern Europe where GPA is more frequently encountered. In Japan, MPA was the predominant subtype (83%), while GPA was more frequent in the United Kingdom (UK) (66%). In terms of the ANCA pattern, >80% of Japanese patients were p-ANCA/ anti-MPO positive, whereas two-thirds of UK patients were c-ANCA/ anti-PR3 positive. Renal involvement in MPA was very common in both countries but was much less common in GPA in Japan compared with the UK [39]. The ANCA subtype may play a role in epidemiology with PR3-ANCA vasculitis more common in northern Europe, northern north America and Australia while MPO-ANCA vasculitis found more in southern Europe, southern United States and Asia [40].

There appears to be a low prevalence of AAV in African populations, with few published papers on ANCA. Adebajo *et al* reported a seroprevalence of 7% for ANA and 30.3% for anti-cardiolipin antibodies amongst healthy west Africans, however no ANCA antibodies were found in this group [25]. This low incidence may be due to a low index of suspicion for these conditions, a lack of appropriate laboratory facilities and relevant medical personnel, resulting in little ANCA testing, or may reflect a true low prevalence amongst black African patients [38][37]. There is inadequate documentation of epidemiology of AAV in the developing world, but there are increasing reports indicating that these conditions are seen in Africa [38].

Anti-neutrophil cytoplasmic antibody testing at Groote Schuur Hospital

The National Health Laboratory Service (NHLS) testing of ANCA in Groote Schuur Hospital (GSH) consists of a fluorescence ELISA designed as a sandwich immunoassay that uses the EliA IgG method on the instrument Phadia 250 [41]. This is an in-vitro qualitative measurement of IgG antibodies directed against PR3 and MPO in human serum [41]. The NHLS state price list as effective from 1st April 2017 is ZAR240.38 for both tests.

Conclusion:

This audit, evaluating the number of ANCA tests ordered, the inappropriateness of each test and the total cost of ANCA testing over 12 months at GSH, will allow better understanding of the negative, positive and false positive ANCA tests. It may demonstrate the need for gatekeeping of the test to strict ordering guidelines with the aim to decrease hospital costs as well as avoid inappropriate specialist clinic

referrals. In addition, the spectrum of AAV in patients attending a tertiary western cape hospital will be studied.

Table 1: 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitis[2]

Large Vessel Vasculitis (LVV)
Takayasu arteritis
Giant cell arteritis
Medium vessel vasculitis
Polyarteritis nodosa (PAN)
Kawasaki disease
Small Vessel Vasculitis
Antineutrophil cytoplasmic antibody associated vasculitis (AAV)
Microscopic polyangiitis (MPA)
Granulomatous with polyangiitis (Wegener's)(GPA)
Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA)
Immune complex SVV
Anti-glomerular basement membrane (anti-GBM) disease
Cryoglobulinaemic vasculitis (CV)
IgA vasculitis (Henoch-Schonlein) (IgAV)
Hypo-complement urticarial vasculitis (HUV) (anti-C1q vasculitis)
Variable vessel vasculitis (VVV)
Bechet's disease
Cogan's syndrome
Single-organ vasculitis (SOV)
Cutaneous leukocytoclastic angiitis
Primary central nervous system
Isolated aortitis
others
Vasculitis associated with systemic disease
Lupus vasculitis
Rheumatoid vasculitis
Sarcoid vasculitis
Others
Vasculitis associated with probable aetiology
Hepatitis C virus-associated cryoglobulinaemic vasculitis
Hepatitis B virus-associated vasculitis
Syphilis-associated aortitis
Drug associated immune complex vasculitis
Drug associated ANCA-associated vasculitis
Cancer-associated vasculitis
Others

Table 2: Clinical indications for anti-neutrophil cytoplasmic antibody testing*[14]

Glomerulonephritis *especially rapidly progressive GN*
Pulmonary haemorrhage *especially pulmonary renal syndrome*
Cutaneous vasculitis with systemic features
Multiple lung nodules
Chronic destructive disease of the upper airways
Long-standing sinusitis or otitis
Subglottic tracheal stenosis
Mononeuritis multiplex or other peripheral neuropathies
Retro-orbital mass
Scleritis

*when there is no other obvious cause

Table 3: Summary of anti-neutrophil cytoplasmic antibody clinical audits worldwide

STUDY	DESCRIPTION	DURATION	TOTAL ANCA	POSITIVE ANCA	%MEETING CLINICAL INDICATIONS	FALSE POSITIVE
Canada, 2018 [21]	Diagnostic outcomes and indications for testing in patients with positive ANCA at a Canadian tertiary care centre	April 2014-March 2015	1889	240	Not assessed	71%
India, 2016 [20]	ANCA testing: Audit from a clinical immunology lab	1 year Jan- Dec 2014	1590	108 (6.8%)	70/108	81/108 85%
The Netherlands, 2016 [19]	Diagnosing ANCA-associated vasculitis in ANCA positive patients	10 years Feb 2005- Feb 2015	8403	1238 (279 pts)	50%	50%
Greece, 2011 [18]	Clinical study ANCA testing in a large cohort of unselected Greek Patients	3 years Sept 2003-Aug 2006	10803	661 (6.1%)	-	439/552 (80%)
New Zealand, 2009 [33]	Appropriateness of ANCA testing in a tertiary hospital	2 years Jan 2006- Dec 2007	1127	118 (9.8%)	376 (33.4%)	-
UK, 2004 [39]	The effect of a symptom related "gating policy" on ANCA requests in routine clinical practice	6 months Jan- Jun 2000	212	57	All were gated requests	20%
Edinburgh, UK, 2001 [40]	The diagnostic value of ANCA testing in a routine clinical setting	4 years April 1996-March 2000	2736	-	-	88%
Georgia, USA, 2001 [41]	ANCA in the absence of Wegener's Granulomatosis or Microscopic Polyangiitis: Implications for the surgical pathologist	7 years 1993-1999	-	247	All respiratory patients with suggestive lung imaging	>90%
Northern Ireland, 1995 [22]	An audit of ANCA in routine clinical practice	45 months Jan 1988 - Oct 1991	-	327	-	220 (73%)
Bristol UK, 1994 [42]	Clinical relevance of testing for ANCA with a standard indirect IF ANCA test in patients with upper or lower respiratory tract symptoms	1990	335	106	-	61(58%)

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CHAPTER 2: PUBLICATION-READY MANUSCRIPT

TITLE PAGE

TITLE:

Anti-neutrophil cytoplasmic antibody (ANCA) testing at Groote Schuur Hospital: Adherence to indications for testing

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None

Abstract

Introduction: Appropriate use of laboratory investigations is increasingly important in resource-constrained environments. We reviewed the anti-neutrophil cytoplasmic antibody (ANCA) testing practices in a tertiary hospital in South Africa.

Methods: We conducted a retrospective file review of all ANCA tests ordered through the National Health Laboratory Services (NHLS) at Groote Schuur Hospital over 12 months (01/01/17 – 31/12/17), including both inpatient and outpatients, and extracted sociodemographic and clinical details. All requests were assessed against the International Consensus Statement of 1999 which provides clinical guidelines for the indications for ANCA testing.

Results: Of the 945 ANCA tests requested, 790 patient records were reviewed, 62 patients had multiple tests and 155 patient records were missing. Most tests (63.5%) were performed on inpatients. Only 193 patients (24.4%) had indications for ANCA testing meeting guidelines. The commonest non-guideline indications were critical limb ischemia (9.6%), stroke (7.3%), uveitis (5.7%), renal impairment (4.9%) and interstitial lung disease (4.4%). The departments for requesting ANCA tests most commonly were Medicine, Ophthalmology, Neurology and Surgery, with Surgery having 99% of its tests for non-guideline indications. Ten patients (1.3%) were diagnosed with ANCA-associated vasculitis (AAV), and of these 9 had renal-limited vasculitis. These patients were predominantly female (70.0%) with mean (SD) age of 54.5 (16.4) years. Twenty-six patients tested ANCA positive without any evidence of AAV. Of these false positives, ten (38.4%) were human immunodeficiency virus (HIV) positive, three (11.5%) had tuberculosis (TB), and three

(11.5%) had other autoimmune diseases. The total estimated cost of ANCA tests for the year was ZAR274 046, with ZAR17 490 spent on duplicate testing and ZAR208 275 spent on non-indicated clinical conditions.

Conclusion: ANCA testing occurred outside standard guidelines in three-quarters of requests, and duplicate testing was common, with large cost implications. Chronic infections HIV and TB, and autoimmune conditions accounted for half of the false positive tests. We believe that training of clinicians and a gating policy will be cost-effective interventions.

Background

Anti-neutrophil cytoplasmic antibodies (ANCA) are integral to the understanding and classification of small vessel vasculitis, and are important diagnostic tools for the ANCA-associated vasculitis (AAV), a group of necrotizing small vessel vasculitis with few or no immune deposits.[1] Because of the high false positive rate for indiscriminate ANCA testing, the International Consensus Statement of 1999 provided clinical guidelines of indications for ANCA testing (Table 1).[2] The aim of these guidelines is to increase the positive predictive value (PPV) of ANCA testing by limiting the test to patients with clinical features suggestive of AAV. It has been shown that ANCA tests have a PPV of 54% and negative predictive value (NPV) of 99% in conventional clinical settings but with a PPV increase to 62% with application of guidelines.[3] Encouragingly, studies show that AAV is seldom missed when testing is restricted to indications listed in the 1999 clinical guidelines.[3]

In African populations, AAV seems to be rare, with few published papers on ANCA antibodies. A combination of a low index of suspicion of AAV and a lack of appropriate laboratory facilities are likely explanations.[4] Amongst 60 west Africans, 6.7% patients with chronic infections (tuberculosis and malaria) were found to be anti-myeloperoxidase (MPO) ANCA positive, but no ANCA antibodies were found amongst healthy Africans.[5]

The rising cost of laboratory investigations, and equitable distribution of health care resources have become important issues world-wide, with both social and political implications. In South Africa (SA), cost-effectiveness is a major criterion in developing clinical policies. We undertook an audit of all ANCA tests ordered in a tertiary academic hospital to determine adherence to indications for testing, the diagnostic accuracy of the test and to review the positive ANCA tests, with the aim of decreasing hospital costs as well as

avoiding inappropriate specialist referrals. The University of Cape Town Faculty of Health Sciences Human Research Ethics Committee approved the study (HREC reference number 443/2018).

Patients and Methods

A retrospective folder review of all ANCA tests ordered through the National Health Laboratory Services (NHLS) was conducted at a state-sector tertiary hospital over 12 months (1/1/2017 – 31/12/2017). Case records were reviewed, and clinico-demographic features, requesting speciality, and comorbidities were collated. The indication for each ANCA test was assessed against the International Consensus Statement of 1999.

The NHLS testing of ANCA was a fluorescence enzyme-linked immunosorbent assay (ELISA) sandwich immunoassay that uses the EliA IgG method on the instrument Phadia 250, measuring IgG antibodies directed against proteinase 3 (PR3) and MPO in human serum.[6]

Statistical analysis

Descriptive statistics and normally distributed variables are presented as means with standard deviations, and sensitivity, specificity, PPV and NPV were calculated. Analysis was done using IBM SPSS Statistics V26.

Results

Of 945 ANCA tests performed, 790 clinical records were found and reviewed, and 155 patient records were missing (Fig. 1). Sixty-two patients had multiple tests with a total of 133 tests ordered. Most tests were ordered for inpatients 498 (63.0%) with 36.5% ordered for

outpatients, and 4 patients (0.5%) having tests from both. Overall, 193 (24.4%) patients had indications which met the 1999 guidelines for ANCA testing. The remaining 597 (75.6%) fell outside the guidelines.

The major requesting departments were Medicine, Ophthalmology, Neurology and Surgery, with Surgery having 99% of its tests for non-guideline indications (Table 2). The commonest non-guideline indications were critical limb ischemia (9.6%), stroke (7.3%), uveitis (5.7%), acute kidney injury (4.9%) and interstitial lung disease (4.4%). The most common guideline indications were glomerulonephritis (GN) (52.1%), peripheral neuropathy (19.0%), scleritis (10.5%) and cutaneous vasculitis (9.0%).

Ten patients (1.3%) were diagnosed with AAV over the 12 months, and these patients were predominantly female, of a mixed racial ancestry with a mean (SD) age of 54.5 (16.4) years (Table 3). One patient was diagnosed with ANCA-negative granulomatosis with polyangiitis (GPA), and 9 had renal-limited vasculitis (RLV) with pauci-immune necrotizing GN on renal biopsy and no extra-renal manifestations. Only five of the RLV patients were ANCA positive (most commonly MPO positive).

Of all ANCA tests performed, 31 (3.9%) patients had positive ANCA tests, of which 5 were true positive tests, and 26 were false postives showing no evidence of AAV. Of these false positives, 11 (42.3%) were associated with chronic infections: human immunodeficiency virus (HIV), tuberculosis (TB), and syphilis, while three (11.5%) had autoimmune diseases (Table 4). No explanation for the positive results were found in 13 patients (50%). The sensitivity, specificity, PPV and NPV for the total cohort were 50.0%, 96.7%, 16.1% and 99.3% respectively.

Of the 193 ANCA tests which met guideline indications for testing, there were 14 patients with positive ANCA tests, of which 5 were true positive tests, and 9 false positives showing no evidence of AAV. When only patients meeting guideline indications for testing were considered, the PPV improved but remained lower than described elsewhere, due to the high number of false positive tests seen in our setting: the sensitivity, specificity, PPV and NPV were 50.0%, 95.1%, 35.7% and 97.2% respectively. The total estimated cost of ANCA tests for the year was ZAR 274 046, with ZAR 17 490 spent on duplicate testing and ZAR 208 275 spent on non-indicated clinical conditions**.**

Discussion

Due to its multi-system heterogenous presentation, insidious onset, and rarity, small vessel vasculitis represents a diagnostic challenge and the ANCA test is an important screening tool. Our study demonstrates that only a quarter of ANCA tests had indications meeting the 1999 international consensus statement guidelines, and this indiscriminate use of ANCA testing is costly, and lowers the test sensitivity and PPV. Elsewhere, similar studies have shown 33.4% and 50.0% of tests were within guideline indications. [7, 8] Gating policies to restrict ANCA testing have proven benefit, reducing false positive rates up to 27.0% [3] and increasing ANCA positivity by 11.8% [9] without missing AAV cases.

False positive ANCA tests are well described, and in the present study resulted in a lower PPV than described elsewhere. [8, 10-12] In our study, there were 26 patients with positive ANCA test and no evidence of AAV, but chronic infection was noted in 42.3%. Elsewhere, chronic infection including TB, malaria, leprosy, suppurative lung disease, infective endocarditis, hepatitis B and C, and HIV have been noted to cause positive ANCA results. [5, 13] In the pre-highly active antiretroviral therapy (HAART) era, Koderisch *et al* described c-ANCA positivity in 24 out of 29 HIV-infected patients (83.0%).[14] More recently, in HIV

patients with mostly good viral control on HAART, 45.0% had at least one autoantibody present, especially ANA (33.0%) and ANCA (13.0%), without clinically relevant disease.[15]

In the present study, 11.5% of the false positive ANCA tests were seen in patients with autoimmune diseases, and this is described elsewhere, particularly amongst patients with chronic autoimmune hepatitis (70.0%), rheumatoid arthritis and systemic lupus erythematosus (20.0%).[16] Other causes of positive results are described, but were not encountered in our study, include medications (anti-thyroid drugs, propylthiouracil, levamisole-adulterated cocaine, minocycline and hydralazine) and inflammatory bowel disease (IBD).[16] The high false positive rate underscores the need to increase the pre-test probability by limiting testing to the 1999 international consensus statement guidelines.

The AAV group was predominantly renal limited vasculitis (RLV), with half these patients having positive ANCA serology. Elsewhere, ANCA-negative RLV has been well documented, highlighting the importance of tissue diagnosis as gold standard when small vessel vasculitis is highly suspected.[17, 18] Poorer renal outcome and less extra-renal involvement are seen in the ANCA negative group.[18]

Limitations of this study include the retrospective folder review where clinician notes that may have inadequately documented the test indication, together with the large number of missing folders. Additionally, this audit was conducted in a tertiary hospital and results are not generalisable to other healthcare platforms.

Conclusions

Our study shows indiscriminate ANCA testing with 75.6% of tests done outside of guideline indications, and duplicate testing was common, with large cost implications. We also demonstrated false positive tests resulting in a lower PPV than described elsewhere. Implementing restrictive protocols for ANCA testing according to the 1999 testing guidelines, together with training of clinicians, is likely to reduce unnecessary tests, resulting in significant cost saving and reduction in inappropriate referrals to sub-specialists.

Table 1: 1999 International Consensus Statement Guidelines of Indications for ANCA Testing[*2]

Glomerulonephritis especially rapidly progressive GN
Pulmonary haemorrhage especially pulmonary renal syndrome
Cutaneous vasculitis with systemic features
Multiple lung nodules
Chronic destructive disease of the upper airways
Long-standing sinusitis or otitis
Subglottic tracheal stenosis
Mononeuritis multiplex or other peripheral neuropathies
Retro-orbital mass
Scleritis

*when there is no other obvious cause

Table 2: ANCA testing according to indication guidelines per department

Department	Total Requests n=790	1999 Guideline Indication No n=598 n (%)	Yes n=192 n (%)	Common non-guideline indications
Cardiology	4	4 (100)	0 (0)	Valvular heart disease
Critical care	22	15 (68.2)	7 (31.8)	AKI, seizures, encephalopathy
Dermatology	10	6 (60.0)	4 (40.0)	Non-specific rash
Otolaryngology	31	27 (87.1)	4 (12.9)	Sensory-neural hearing loss, vocal cord paralysis
Gastroenterology	3	3 (100)	0 (0)	IBD
General Medicine	272	173 (63.6)	99 (36.4)	AKI, Stroke
Nephrology	30	15 (50.0)	15 (50.0)	AKI
Neurology	100	70 (70.0)	30 (30.0)	Stroke, encephalopathy
OBGYN	25	20 (80.0)	5 (20.0)	Recurrent pregnancy losses, POF
Ophthalmology	105	83 (79.1)	22 (20.9)	Uveitis, optic neuritis
Orthopaedics	28	28 (100)	0 (0)	Trigger finger, CTS, arthritis
Psychiatry	2	2 (100)	0 (0)	Encephalopathy, psychosis
Pulmonology	35	32 (91.4)	3 (8.6)	ILD, poorly controlled asthma, PHT
Rheumatology	25	23 (92.0)	2 (8.0)	Raynaud's phenomenon, arthritis
Surgery	95	94 (98.9)	1 (1.1)	CLI, vascular aneurysms
Trauma	3	3 (100)	0 (0)	ICA dissection, TBI

Abbreviations: OBGYN: Obstetrics and gynaecology; AKI: Acute kidney injury; POF: premature ovarian failure; IBD: Inflammatory bowel disease; CTS: Carpal tunnel syndrome; ILD: Interstitial lung disease; PHT: Pulmonary hypertension; CLI: Critical limb ischemia; ICA: Internal carotid artery; TBI: Traumatic brain injury

Table 3: Details of ten patients with anti-neutrophil cytoplasmic antibody-associated vasculitis

Gender	Age	Ethnicity	Department	AAV type	MPO	PR3	Guideline Indication
M	55	Mixed	Medicine	GPA	Neg	Neg	Yes
F	49	Mixed	Medicine	RLV	Pos	Neg	Yes
F	37	Mixed	Medicine	RLV	Pos	Neg	Yes
M	81	Caucasian	Medicine	RLV	Pos	Neg	Yes
F	71	Mixed	Medicine	RLV	Pos	Neg	Yes
M	39	Mixed	Medicine, Nephrology	RLV	Neg	Pos	Yes
F	58	African	Medicine, Nephrology	RLV	Neg	Neg	Yes
F	58	Mixed	Nephrology	RLV	Neg	Neg	Yes
F	29	Mixed	Nephrology	RLV	Neg	Neg	Yes
F	68	Mixed	Medicine	RLV	Neg	Neg	Yes

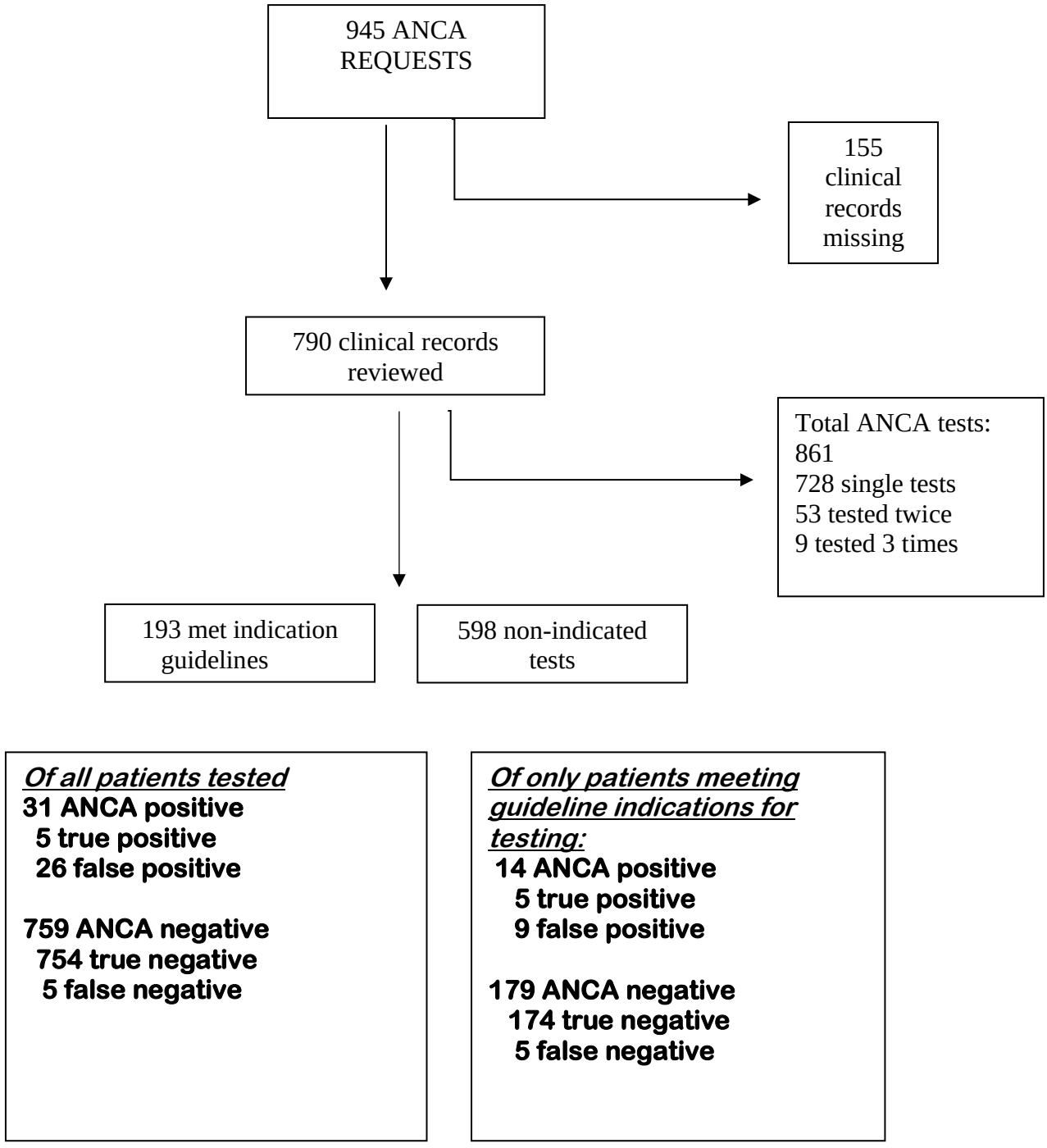
Abbreviations: M: male; F: female; AAV: anti-neutrophil cytoplasmic antibody-associated vasculitis; MPO: anti-myeloperoxidase antibody; PR3: anti-proteinase 3 antibody; Pos: positive; Neg: negative; RLV: renal limited vasculitis

Table 4: Positive anti-neutrophil cytoplasmic antibody results in patients with no evidence of anti-neutophil cytoplasmic antibody-associated vasculitis

M/F	Age	Ethnicity	MPO	PR3	Guideline indication	Indication description	Clinical information
M	47	African	Pos	Neg	Yes	GN	HIV, AKI due to hypertension nephrosclerosis
F	48	African	Pos	Neg	Yes	GN	HIV, HIVAN
F	43	African	Pos	Neg	No	hepatitis	HIV, confirmed DILI
F	33	Mixed	Neg	Pos	No	Hepatomegaly and lymphadenopathy	HIV, HIV seroconversion
M	47	Mixed	Neg	Pos	No	cholangitis	HIV, syphilis, confirmed liver abscesses
F	44	African	Pos	Pos	Yes	Palpable purpura	HIV, biopsy- cutaneous lupus
M	35	African	Neg	Pos	Yes	GN	TB, AKI and macroscopic haematuria
F	38	African	Pos	neg	No	AKI	HIV, disseminated TB
F	30	African	Pos	Neg	Yes	PN	HIV, TB
M	39	African	Pos	Neg	No	Valve heart disease	HIV, TB, epilepsy
F	57	Mixed	Neg	Pos	No	seizures	HPT, syphilis
F	80	Mixed	Pos	Neg	No	ILD	RA, HPT, hyperthyroidism
M	38	Caucasian	Pos	Neg	No	hepatitis	AIH
F	55	African	Pos	Neg	No	Unclear	SLE
M	67	Mixed	Pos	Neg	Yes	GN	HPT, biopsy- diffuse glomerulosclerosis
F	69	Mixed	Pos	Neg	Yes	GN	HPT, DM, asthma, biopsy- MCGN and ATN
M	47	African	Pos	Neg	No	AKI	-
F	62	Mixed	Pos	Neg	Yes	PN	HPT, ILD
F	68	Mixed	Pos	Neg	No	myelopathy	HPT
F	73	Mixed	Pos	Neg	No	ILD	HPT, DM
F	54	Mixed	Pos	Neg	No	Poorly controlled asthma	HPT, asthma
M	29	African	Pos	Pos	No	uveitis	-
M	66	Mixed	Neg	Pos	Yes	scleritis	HPT
M	41	Asian	Neg	Pos	No	amblyopia	-
F	48	Mixed	Neg	Pos	No	Raynaud's phenomenon	Breast cancer in remission
F	38	African	Pos	Neg	No	POF	-

Abbreviations: M: Male; F: Female; MPO: anti-myeloperoxidase antibodies; PR3: anti-proteinase 3 antibodies; Neg: negative; Pos: positive; GN: glomerulonephritis; AKI: acute kidney injury; PN: peripheral neuropathy; ILD: interstitial lung disease; HIV: human immuno-deficiency virus; HIVAN: HIV associated nephropathy; TB: tuberculosis; SLE: systemic lupus erythematosus; LN: lupus nephritis; HPT: hypertension; AIH: autoimmune hepatitis; RA: rheumatoid arthritis; DM: diabetes mellitus; DILI: drug induced liver injury; MCGN: mesangio-capillary glomerulonephritis; ATN: acute tubular necrosis; POF: premature ovarian failure

Fig. 1. Flow diagram of anti-neutrophil cytoplasmic antibodies performed at a tertiary hospital in South Africa



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Appendix 1: Data Capture Sheet

NAME:			
FOLDER NUMBER:			
DATE OF BIRTH:			
MALE		**FEMALE**	
CAUCASIAN		**AFRICAN**	
COMORBIDITIES			
HIV		HEP B	
TB		HEP C	
Infective endocarditis			
Other:			
DEPARTMENT			
INPATIENT		OUTPATIENT	

INDICATIONS

EYES			
Retro-orbital mass		Uveitis	
Scleritis			
Other:			
ENT			
Upper Airway destruction		Otitis	1 or more
Nasal perforation		Sinusitis	1 or more
Subglottic stenosis			
Other:			
CNS			
Mononeuritis Multiplex		Stroke	
Peripheral neuropathy			
Other			

RESPIRATORY			
Pulmonary haemorrhage: haemoptysis Imaging			
Lung nodules		Interstitial lung disease	
Biopsy			
Other:			
RENAL: GLOMERULONEPHRITIS			
haematuria		Red cell casts	
proteinuria		Cresenteric Glomerulonephritis	
Other			
SKIN			
Purpura (palpable)		Urticaria papules	
nodules		Ulcers	
Levido reticularis		Necrosis	
Skin biopsy			
Other:			
GIT			
Autoimmune hepatitis		Inflammatory bowel disease	
Primary Sclerosing cholangitis			
Other:			
CONNECTIVE TISSUE DISEASE			
Systemic lupus erythematosus		Scleroderma	
Rheumatoid arthritis			
Other:			
KNOWN ANCA ASSOCIATED VASCULITIS			
GPA/Wegeners		EGPA/Churg strauss	
MPA		Pauciimmune GN	
FOLLOW UP OF PREVIOUSLY POSITIVE ANCA			
DRUGS			

propylthiouracil		sulphasalazine	
hydralazine		Clozapine	
phenytoin		allopurinol	
Other indications:			

ANCA RESULTS			
Test 1			
P-ANCA	C-ANCA	MPO	PR3
Test 2			
P-ANCA	C-ANCA	MPO	PR3
Test 3			
P-ANCA	C-ANCA	MPO	PR3
Test 4			
P-ANCA	C-ANCA	MPO	PR3

Appendix 2: UCT Ethics Approval Letter

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS011: Study deviation

HREC office use only (FWA00001637; IRB00001938)			
This serves as acknowledgement of a protocol deviation as described below			
Chairperson of the HREC signature	Signature removed	Date	30/8/19

Principal Investigator to complete the following:

1. Protocol Information

Date (when submitting this form)	29/08/19
HREC REF Number	443/2018
Project Title	Anti-neutrophil cytoplasmic antibody (ANCA) testing at Groote Schuur Hospital: Adherence to indications for testing over a 12 month period
Protocol number (if applicable)	
Principal Investigator	Prof B Hodkinson (MMED student: Dr Ramona Govender)
Department / Office Internal Mail Address	drbridget@gmail.com (ramona.govender3@gmail.com)

2. Protocol deviation description

Please describe the deviation below, including **the reason** why **the deviation occurred.**

Late renewal of ethics/ annual progress report.

The renewal date was missed on account of the MMED student writing final examinations.

3. Follow-up actions

3.1 Please describe any follow-up **action(s) taken or planned as a result of this deviation e.g. DSMB reporting,** report **to** sponsor, informing participants.

The study is for degree purpose and does not involve any sponsors or DSMB reporting. There are also participants involved.

3.2 Please describe what action(s) have or will be taken to prevent similar deviations in future.

We aim to complete this project in the next 12 months and we don't expect to overrun this period. We have also placed reminders if needed.

4. Principal Investigator's acknowledgement of responsibility

This **signature** indicates **the** PI **has** reviewed **the deviation, taken appropriate** follow-up **action and implemented or plans to implement preventative steps where possible.**

Signature of PI	Signature removed	Date	**29/08/19**

Appendix 3: GSH Permission Letter

Western Cape Government
Health

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
E-mail : Bernadette.Eick@westerncape.gov.za

Professor B. Hodkinson
MEDICINE - RHEUMATOLOGY

E-mail: drbridget@gmail.com / ramona.govender3@gmail.com

Dear Professor Hodkinson,

RESEARCH PROJECT EXTENSION: Anti-Neutrophil Cytoplasmic Antibody (ANCA) Testing At Groote Schuur Hospital: Adherence To Indications For Testing Over A 12-Month Period

Your recent communication to the hospital refers.

The extension of your research is approved in accordance with UCT Ethics clearance, until **30 August 2020.**

As previously mentioned:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assst with the research.
c) No additional costs to the Hospital should be incurred i.e. Lab, consumables or stationary. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form.**
d) No patient folders may be removed from the premises or be inaccessible.
e) **No patient folders may be removed from the premises or be inaccessible.**
f) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
g) Confidentiality must be maintained at all times.
h) Once the research is complete, please submit a copy of the publication or report.

I would like to wish you every success with the project.

Yours sincerely

Signature removed

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 2 October 2019

C.C. Mr L. Naidoo
Dr L. Booyens
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Appendix 4: Instructions to the author from South African Medical Journal (SAMJ)

Author Guidelines

The *SAMJ* has launched a new submission and tracking system. Authors will be required to register a profile on the Editorial Manager platform in order to submit a manuscript.

To submit a manuscript, please proceed to the *SAMJ* Editorial Manager website:

www.editorialmanager.com/samj

To access and submit an article already in production, please see the guidelines here.

Author Guidelines

Please view the Author Tutorial for guidance on how to submit on Editorial Manager.

Please take the time to familiarise yourself with the policies and processes below. If you still have any questions, please do not hesitate to ask our editorial staff (tel.: +27 (0)21 532 1281, email: submissions@hmpg.co.za).

SAMJ policies

- Types of articles considered by the SAMJ
- Article Processing Charges
- Authorship
- Conflict of interest
- Research ethics committee approval
- Clinical trials
- Protection of patient's rights to privacy
- Copyright notice
- Privacy statement
- Ethnic classification
- CPD

Manuscript preparation

- Preparing an article for anonymous review
- General article format/layout
- Preparation notes by article type
- Illustrations
- Tables
- References

From submission to acceptance

- Submission and peer-review
- Production process
- Changing contact details or authorship

Publication

- Online versus print
- Errata and retractions
- Indexing

SAMJ Policies

Type of articles considered by the SAMJ

The *SAMJ* will no longer limit the articles accepted to those that have 'general medical content', but is intending to capture the spectrum of medical and health sciences, grouped by relevance to the country's burdens of disease. This content will include research in the social sciences and economics that is relevant to the medical issues around our burden of disease. Please see 'A new vision for the *SAMJ* – and a call for papers' for a full discussion of the new directions for the *SAMJ*.

We accept the following types of articles:

Research
Reviews
Clinical trials
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In Practice (Previously Forum incl. Case Reports)
Correspondence
Obituaries
Book reviews
Ad hoc supplements e.g. guidelines, conference/congress abstracts, Festschrifts*

The following articles are by invitation only:

Guest editorial
Continuing Medical Education (CME)

*Contact claudian@hmpg.co.za for information on submitting ad hoc/commissioned supplements, including guidelines, conference/congress abstracts, Festschrifts, etc.

Publication Fees

All articles published in the *South African Medical Journa*l are open access and freely available online upon publication. This is made possible by applying a business model to offset the costs of peer review management, copyediting, design and production, by charging a publication fee of R5 565 (ex vat) for each research article published. The charge applies only to **Research** articles submitted after 1 March 2017. The publication fee is standard and does not vary based on length, colour, figures, or other elements.

When submitting a Research article to the *SAMJ*, the submitting author must agree to pay the publication fee should the article be accepted for publication. The publicaiton fee is payable when your manuscript is editorially accepted and before production commences for publication. The submitting author will be notified that payment is due and given details on the available methods of payment. Prompt payment is advised; the article will not enter into production until payment is received.
Queries can be directed to claudian@hmpg.co.za.

Please refer to the section on 'Sponsored Supplements' regarding the publication of

supplements, where a charge is applicable. Queries can be directed to dianes@hmpg.co.za or claudian@hmpg.co.za

Authorship

Named authors must consent to publication. Authorship should be based on: (*i*) substantial contribution to conceptualisation, design, analysis and interpretation of data; (*ii*) drafting or critical revision of important scientific content; or (*iii*) approval of the version to be published. These conditions must all be met (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org)

If authors' names are added or deleted after submission of an article, or the order of the names is changed, all authors must agree to this in writing.

Please note that co-authors will be requested to verify their contribution upon submission. Non-verification may lead to delays in the processing of submissions.

Author contributions should be listed/described in the manuscript.

Conflicts of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. The existence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest. If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Research ethics committee approval

Authors must provide evidence of Research Ethics Committee approval of the research where relevant. Ensure the correct, full ethics committee name and reference number is included in the manuscript.

If the study was carried out using data from provincial healthcare facilities, or required active data collection through facility visits or staff interviews, approval should be sought from the relevant provincial authorities. For South African authors, please refer to the guidelines for submission to the National Health Research Database. Research involving human subjects must be conducted according to the principles outlined in the Declaration of Helsinki. Please refer to the National Department of Health's guideline on Ethics in Health research: principles, processes and structuresto ensure that the appropriate requirements for conducting research have been met, and that the HPCSA's General Ethical Guidelines for Health Researchers have been adhered to.

Clinical trials

As per the recommendations published by the International Committee of Medical Journal Editors (ICMJE), clinical trial research is any research that assigns individuals to an intervention, with or without a concurrent comparison/control group to study the cause-and-effect relationship between the intervention and health outcomes. All clinical trials should be registered with the appropriate national clinical trial registry (or any international primary register, if relevant), and the trial registration number should be cited at the end of the

abstract. All clinical trial reports must also contain a data sharing statement as per the recommendations of the ICMJE. Statements are to indicate:

- whether individual deidentified participant data will be shared;
- what data in particular will be shared; whether additional, related documents will be available;
- when the data will become available and for how long; by what access criteria data will be shared.

Please see the ICJME announcement for further details and illustrative examples of data sharing statements: ICMJE Data Sharing Statements for Clinical Trials

Since 1st December 2005, all clinical trials conducted in South Africa have been required to be registered in the South African National Clinical Trials Register. The SAMJ therefore requires that clinical trials be registered in the relevant public trials registry at or before the time of first patient enrollment as a condition for publication. The trial registry name and registration number must be included in the manuscript.

Please refer to the general guidelines for all papers at the top of this article for additional requirements with respect to ethics approval, funding, author contributions, etc. The format of original research articles should be followed for reporting of clinical trial results.

Patient Consent

Information that would enable identification of individual patients should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) has given informed written consent for publication and distribution. We further recommend that the published article is disseminated not only to the involved researchers but also to the patients/participants from whom the data was drawn. Refer to Protection of Research Participants. The signed consent form should be submitted with the manuscript to enable verification by the editorial team.

Other individuals

Any individual who is identifiable in an image must provide written agreement that the image may be used in that context in the *SAMJ.*

Copyright notice

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Material submitted for publication in the *SAMJ* is accepted provided it has not been published or submitted for publication elsewhere. Please inform the editorial team if the main findings of your paper have been presented at a conference and published in abstract form, to avoid copyright infringement. All research already published as 'Conference proceedings' needs to be substantially re-written, with a new title, a new abstract and new and important results to back up any study before it will be considered for a new publication. The *SAMJ* does not hold itself responsible for statements made by the authors.

Previously published images

If an image/figure has been previously published, permission to reproduce or alter it must be obtained by the authors from the original publisher and the figure legend must give full

credit to the original source. This credit should be accompanied by a letter indicating that permission to reproduce the image has been granted to the author/s. This letter should be uploaded as a supplementary file during submission.

Privacy statement

The *SAMJ* is committed to protecting the privacy of its website and submission system users. The names, personal particulars and email addresses entered in the website or submission system will not be made available to third parties without the user's permission or due process. By registering to use the website or submission system, users consent to receive communication from the *SAMJ* or its publisher HMPG on matters relating to the journal or associated publications. Queries with regard to privacy may be directed to publishing@hmpg.co.za.

Ethnic/race classification

Use of racial or ethnicity classifications in research is fraught with problems. If you choose to use a research design that involves classification of participants based on race or ethnicity, or discuss issues with reference to such classifications, please ensure that you include a detailed rationale for doing so, ensure that the categories you describe are carefully defined, and that socioeconomic, cultural and lifestyle variables that may underlie perceived racial disparities are appropriately controlled for. Please also clearly specify whether race or ethnicity is classified as reported by the patient (self-identifying) or as perceived by the investigators. Please note that is not appropriate to use self-reported or investigator-assigned racial or ethnic categories for genetic studies.

Continuing Professional Development (CPD)

SAMJ is an HPCSA-accredited service provider of CPD materials. Principal authors can earn up to 15 CPD continuing education units (CEUs) for publishing an article; co-authors are eligible to earn up to 5 CEUs; and reviewers of articles can earn 3 CEUs. Each month, *SAMJ* also publishes a CPD-accredited questionnaire relating to the academic content of the journal. Successful completion of the questionnaire with a pass rate of 70% will earn the reader 3 CEUs. Administration of our CPD programme is managed by Medical Practice Consulting. To complete questionnaires and obtain certificates, please visit MRP Consulting

Manuscript preparation

Preparing an article for anonymous review

To ensure a fair and unbiased review process, all submissions are to include an anonymised version of the manuscript. The exceptions to this are Correspondence, Book reviews and Obituary submissions.

Submitting a manuscript that needs additional blinding can slow down your review process, so please be sure to follow these simple guidelines as much as possible:

- An anonymous version should not contain any author, affiliation or particular institutional details that will enable identification.
- Please remove title page, acknowledgements, contact details, funding grants to a named person, and any running headers of author names.
- Mask self-citations by referring to your own work in third person.

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

- Research

- Editorials
- CME
- In Practice and Case reports
- Reviews
- Clinical trials
- Correspondence
- Obituaries
- Book reviews
- Guidelines

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Here is an example of a good abstract.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Editorials

Guideline word limit: 1 000 words

These opinion or comment articles are usually commissioned but we are happy to consider and peer review unsolicited editorials. Editorials should be accessible and interesting to readers without specialist knowledge of the subject under discussion and should have an element of topicality (why is a comment on this issue relevant now?) There should be a clear message to the piece, supported by evidence.

Please make clear the type of evidence that supports each key statement, e.g.:

- expert opinion
- personal clinical experience
- observational studies
- trials
- systematic reviews.

CME (by invite only)

CME is intended to provide readers with practical, up-to-date information on medical and related matters. It is aimed at those who are not specialists in the field.

From January 2016, all CME articles will be printed in full in the *SAMJ*. Please try to adhere strictly to the guidelines on word count as we have a page limit for the print issue of the *SAMJ*. We reserve the right to place some tables and reference lists online if this is necessary for space.

In practice, this means that each CME topic usually covers two issues of the print issue of the *SAMJ*.

The guest editor, in consultation with the editor, is responsible for convening a team of authors, deciding on the subjects to be covered and for reviewing the manuscripts submitted. The suggestion is for 4 - 5 articles, although there is some room for flexibility contingent on discussions with the editor.

For queries about these guidelines please feel free to contact the CME editor, Dr Bridget Farham, by email (ugqirha@iafrica.com) or telephone (+27 (0)82 452 2860)

Review process

The guest editor reviews the articles and returns them to the CME editor for review and final approval.

Guest editorials

Guideline word limit: 1 000 words

- Include the guest editor's personal details (qualifications, positions, affiliation, e-mail address, and a short personal profile (50words)).
- If possible, include a photograph of the author(s) at high enough resolution for print. It is preferable to provide two guest editorials, one for each issue, so that the content of the articles in each issue is covered.

Articles

Guideline word limit: 2 000 - 3 000 words

- Each article requires an abstract of ±200 words.
- The editor reserves the right to shorten articles but will send a substantially shortened article back for author approval.

Personal details

Please supply: Your qualifications, position and affiliations and MP number (used for CPD points); Address, telephone number and fax number, and your e-mail address; and a short personal profile (50words)and a few words about your current fields of interest.

In Practice

Guideline word limit: 2 000 - 3 000words

This section includes articles that would previously have been accepted into the Forum section, and case reports.

In practice articles are those that draw attention to specific issues of clinical, economic or political interest regarding medicine and healthcare in southern Africa. They are assigned to a topic:

Case report
Clinical practice
Clinical alert
Issues in medicine
Issues in public health
Healthcare delivery
Medicine and the environment
Medicine and the law
Cochrane corner

An In Practice article should follow the following format – sub-headings are not necessary, but may be used for clarity:

- Author affiliations and qualifications: to be the same as for Research. Provide all authors' names and initials, qualifications and full affiliations, and corresponding author.
- Short abstract: does not need to be structured, but should capture the essential features of the article
- Introduction: the reason for the article and the issue being addressed
- Recent research, discussion, local policy around the issue – include your own research where appropriate
- All statements should be referenced and, if opinion only, this should be stated
- Discussion: how this article adds to the discussion around a particular topic
- If a clinical practice or policy point is at issue, this needs to be emphasised, using a box with highlights if appropriate.

Essentially In practice is an opportunity for a more discursive approach to topics of clinical, economic or political importance in southern African health systems. It is not an opportunity to put forward unsubstantiated opinions!

Case reports

The *SAMJ* has recently started to accept case reports. The cases must come from Africa, preferably southern Africa unless the condition is common to all African countries, and must be either a completely new description of a clinical condition or result (use Google!) or a case that highlights important practice or management issues.

Please use the following format for case reports:

- Title of case: do not include the words 'a case report' in the title
- Summary/abstract: up to 150 words summarising the case presentation and outcome
- Background: why is this case important and why did you write it up?
- Case presentation: presenting features, medical, social, family history as appropriate
- Case management: should be according to best practice, and if not, please explain why
- Investigations, if relevant: save space by simply saying 'normal' if, for example, renal function was completely normal, rather than listing normal results, highlight the abnormal – or indeed the normal if this is clinically significant
- Differential diagnosis, if relevant
- Treatment, if relevant
- Outcome and follow-up
- Discussion – a VERY BRIEF review of similar published cases
- Teaching points: 3 - 5 bullet points
- References: as per the *SAMJ* house style
- Tables and figures: keep to a minimum. Use clinical images where relevant – we need hi-res versions for print, and identifiable persons must have a consent form
- Patient consent: please include a statement about patient consent to a written case report. This should be uploaded as a supplementary file.

Clinical trials

Guideline word limit: 4000 words

As per the recommendations published by the International Committee of Medical Journal Editors (ICMJE), clinical trial research is any research that assigns individuals to an intervention, with or without a concurrent comparison/control group to study the cause-and-effect relationship between the intervention and health outcomes. All clinical trials should be registered with the appropriate national clinical trial registry (or any international primary register, if relevant), and the trial registration number should be cited at the end of the abstract. Since 1st December 2005, all clinical trials conducted in South Africa have been required to be registered in the South African National Clinical Trials Register. The *SAMJ*therefore requires that clinical trials be registered in the relevant public trials registry at or before the time of first patient enrollment as a condition for publication. The trial registry name and registration number must be included in the manuscript.

Please refer to the general guidelines for all papers at the top of this article for additional requirements with respect to ethics approval, funding, author contributions, etc. The format of original research articles should be followed for reporting of clinical trial results.

Review articles

Guideline word limit: 4 000 words

These are welcome, but should be either commissioned or discussed with the Editor before submission. A review article should provide a clear, up-to-date account of the topic and be aimed at non-specialist hospital doctors and general practitioners.

Please ensure that your article includes:

- Abstract: unstructured, of about 100-150 words, explaining the review and why it is important
- Methods: Outline the sources and selection methods, including search strategy and keywords used for identifying references from online bibliographic databases. Discuss the quality of evidence.
- When writing: clarify the evidence you used for key statements and the strength of the evidence. Do not present statements or opinions without such evidence, or if you have to,

say that there is little or no evidence and that this is opinion. Avoid specialist jargon and abbreviations, and provide advice specific to southern Africa.

- Personal details: Please supply your qualifications, position and affiliations and MP number (used for CPD points); address, telephone number and fax number, and your e-mail address; and a short personal profile (50 words) and a few words about your current fields of interest.

Correspondence (Letters to the Editor)

Guideline word limit: 500 words

Letters to the editor should relate either to a paper or article published by the SAMJ or to a topical issue of particular relevance to the journal's readership

- May include only one illustration or table
- Must include a correspondence address.

Book reviews

Guideline word limit: 400 words

Should be about 400 words and must be accompanied by the publication details of the book. Provide a hi-res image of the cover if possible (with permission from the copyright holder).

Obituaries

Guideline word limit: 400 words

Should be offered within the first year of the practitioner's death, and may be accompanied by a photograph.

Guidelines

Guidelines should always be discussed with the Editor prior to submission.

Because of the intensive review process required to ensure Guidelines are independent, evidence-based and free from commercial bias, they are usually published as a supplement to the *SAMJ*, the costs of which must be covered by sponsorship, advertising or payment by the guideline authors/association. We will provide a quote based on the expected length of the guideline and whether it is to appear online only, or in print, which must be accepted by the body putting the guidelines together before submitting the work to the SAMJ.

The Editor reserves the right to determine the scheduling of supplements. Understandably, a delay in publication must be anticipated dependent upon editorial workflow.

All guidelines should include a clear, transparent statement about all sources of funding and an explicit, clear statement of conflicts of interest of any of the participants in the guidelines about industry funding for lectures, research, conference participation etc.

All guidelines should be structured according to Agree II.

Please access this website before putting the guidelines together, download the Agree 11 instrument and use this to put the guidelines together.

All submitted guidelines will be sent to the local Agree II appraisal committee for review and must be endorsed by an appropriate body prior to consideration and all conflicts of interest expressed.

A structured abstract not exceeding 400 words (recommended sub-headings: *Background, Recommendations, Conclusion*) is required. Sections and sub-sections must be numbered

consecutively (e.g. 1. Introduction; 1.1 Definitions; 2.etc.) and summarised in a Table of Contents.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:
Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:
Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:
Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the Crossref homepage, paste the article title into the 'Metadata search' box.
  - Look for the correct, matching article in the list of results.
  - Click Actions > Cite
  - Alongside 'url =' copy the URL between { }.
  - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx.doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references

  - Government Gazettes:

  National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

  In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

  - Provincial Gazettes:

  Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

  - Acts:

  South Africa. National Health Act No. 61 of 2003.

  - Regulations to an Act:

South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

Rex v Jopp and Another 1949 (4) SA 11 (N)

Rex v Jopp and Another: Name of the parties concerned

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports

11: Page or section number

(N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

NOTE: no . after the v

- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.

From submission to acceptance

Submission and peer-review

To submit an article:

- Please ensure that you have prepared your manuscript in line with the SAMJ requirements.
- All submissions should be submitted via Editorial Manager
- The following are required for your submission to be complete:
  - Anonymous manuscript (unless otherwise stated)
  - Manuscript
  - Any supplementary files: figures, datasets, patient consent form, permissions for published images, etc.
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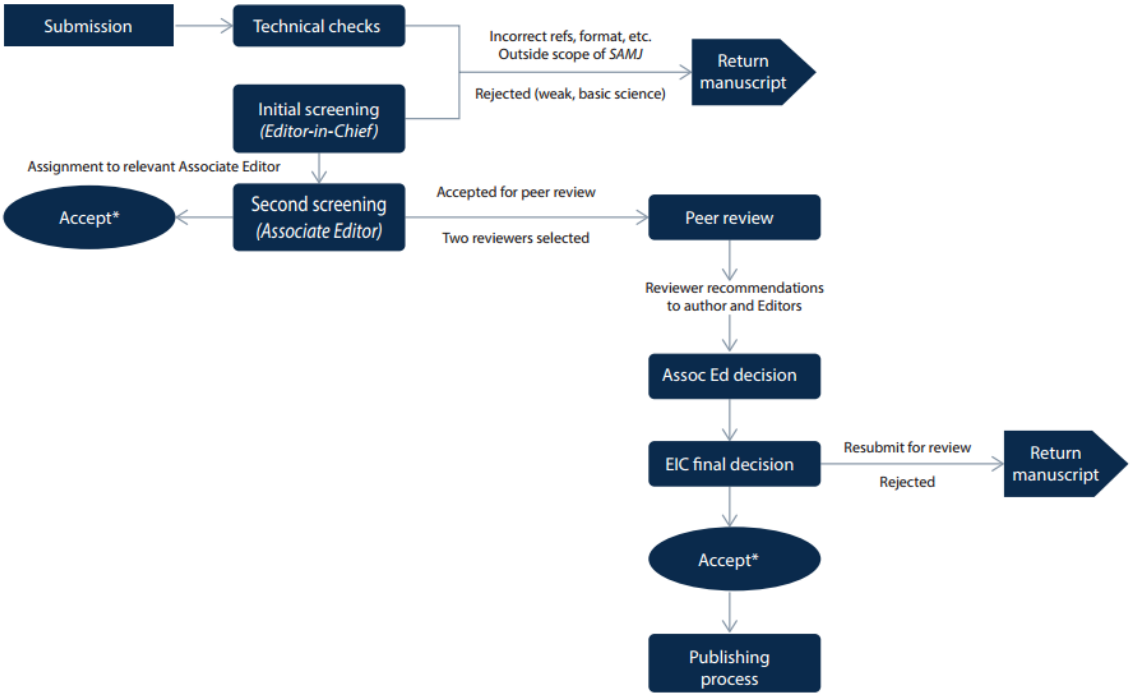
Peer-review process



*Manuscripts accepted at this point are limited to Editorials, Correspondence, Obituaries, Book reviews, Abstracts, CME
**Some minor revisions may be requested

Production process

Please note that there is a 6-month waiting time for publication, once an article has been sent to the production team.

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2. The CE copyedits in Word, working on house style, format, spelling/grammar/punctuation, sense and consistency, and preparation for typesetting.
3. If the CE has an author queries, he/she will contact the corresponding author and send them the copyedited Word doc, asking them to solve the queries by means of track changes or comment boxes.
4. The authors are typically asked to respond within 1-3 days. Any comments/changes must be clearly indicated e.g. by means of track changes. Do not work in the original manuscript - work in the copyedited file sent to you and make your changes clear.
5. The CE will finalise the article and then it will be typeset.
6. Once typeset, the CE will send a PDF of the file to the authors to complete their final check, while simultaneously sending to the 2nd-eye proofreader.
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8. The CE implements the authors' and proofreader's mark-ups, finalises the file, and prepares it for the upcoming issue.

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